Optical Nanosensors for Chemical Analysis and Imaging Outside and Inside Single Living Cells

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We have developed a method for minimally invasive intracellular chemical analysis, employing a nanometer scale optical device that can be entirely inserted into a single living cell. These devices consist of monodisperse spherical 20-200 nm PEBBLE (Probe Encapsulated by Biologically Localized Embedding) sensors, which are composed of a polymer matrix incorporated with fluorescent indicator dyes, and have been developed for several analytes, including calcium, pH, potassium and oxygen. The fluorescent PEBBLE sensors have been made ratiometric, show good reversibility and are biocompatible. Protein binding and other features of the local cell environment do not create erroneous readings, making it possible to calibrate the sensors outside of the cell. PEBBLE sensors have been delivered in several ways, including gene gun bombardment and packaging in liposomes, and are used with traditional optical microscopy and spectroscopy, as well as with time resolved fluorescence and confocal microscopy. Furthermore, as an individual PEBBLE can be imaged (though not resolved) by a microscope, autofluorescence becomes negligible.

We note that one nanopebble takes up only 1 PPM of the volume of a 2 micron bacteria. However, despite their small size, PEBBLE sensors were found to be highly stable, both physically (including pressure) and photophysically. The effects of photobleaching are minimized by using ratiometric or lifetime measurements and short exposure times (100 ms or less). The PEBBLE response time is 1 ms or less. The pH sensitivity is 0.01 and PEBBLEs have been prepared for all biological pH ranges. The calcium sensitivity is near 1 nM and the potassium PEBBLEs are highly selective against sodium (3 orders of magnitude). Using a universal ion-correlation approach, we have also developed nanosensors for other ions, such as sodium, magnesium, chloride and nitrite. We note that while no free dye indicators exist for glucose or nitrite (and for many other ions), fluorescent nanosensors have been made for these analytes, and others are in preparation, including voltage (and ionic strength) PEBBLE sensors. The fiber-optic nanosensors (100 nM) we developed have most of the advantages of the PEBBLE sensors: biocompatibility, controlled nanoenvironment (allowing highly quantitative calibration), and non interference among sensors, which allows simultaneous parallel processing of many different analytes (Ca, K, pH, oxygen, etc.).

Both fiber optic and PEBBLE nanosensors have been applied to a variety of experimental biological models of disease. Fiber optic nanobiosensors inserted into mouse oocytes, rat pulmonary macrophages, human SY5Y neuroblastoma or glioma cells in culture have provided real-time physiological data on alteration of intracellular calcium, pH and potassium following a variety of pathogenic and toxic stimuli. Up to two fiber sensors have been simultaneously introduced into a single living cell with excellent spatial and temporal resolution of ionic fluxes. Use of PEBBLE sensors has obviated the need for the introduction of multiple fiber sensors into a single cell. Submicron resolution of PEBBLEs containing a locator dye and a calcium sensing dye provide physiological readout without dye-dye or cell-sensor interference. Similar spatio-temporal resolution has been achieved with combinations of PEBBLEs that monitor pH, O2, K+, Cl-, and other analytes in vitro. Optical nanosensors have also proven useful in the spectrofluorometric measurement and imaging of calcium fluxes from isolated cellular organelles. PEBBLE sensors show increased sensitivity and diminished sample interference, when compared with soluble calcium-sensing dyes. Both fiber-based and PEBBLE nanosensors have demonstrated utility in the minimally invasive measurement of physiologic change in real

time. Optical nanosensors increase the ability of investigators to probe nanodomains within living cells for more accurate assessment of critical components of pathogenic cascades.